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Plant quality and primary productivity modulate plant biomass responses to the joint effects of grazing and fertilization in a mesic grassland

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Abstract

Questions: Human activities are increasing the density of domestic grazers and global nutrient loads, modifying the main determinants of vegetation community dynamics. Grazing (top-down control) and nutrient availability (bottom-up control) may interactively modify plant biomass, which is particularly important in grasslands devoted to livestock production. Here, we aim to understand the interactive effects of grazing and fertilization on grassland plant biomass. We hypothesized that the joint effects of nutrient addition and domestic grazing on above-ground plant biomass are not additive, but they modify each other through changes in ground-level light, leaf nutritional quality, above-ground net primary productivity (ANPP), and below-ground plant allocation.

Location: Flooding Pampa (Buenos Aires, Argentina).

Methods: We carried out a factorial experiment of grazing exclusion and fertilization with nitrogen, phosphorus, and potassium + micronutrients during several years in a mesic grassland devoted to livestock production.

Results: After four years, grazing reduced live above-ground plant biomass by 52%, and when combined with fertilization this reduction was 70%. Nutrient addition in the grazed grassland increased ANPP and leaf nutrient concentration. These changes in turn intensified grazing pressure and cattle's plant consumption. By contrast, fertilization did not produce any significant effect on plant biomass or ANPP inside the exclosures, where ground-level light was low. A structural equation model revealed that the increase in ANPP fostered above-ground and reduced below-ground plant biomass.

Conclusions: This is the first study conducted in the Pampas grasslands that evaluated the effect of cattle grazing and fertilization on plant communities under field conditions over several years. Grazing and nutrient addition synergistically controlled grassland plant biomass, as the reduction in above-ground biomass by cattle consumption was greater in fertilized plots. Our results provided empirical evidence that leaf nitrogen and ANPP modulated plant biomass dynamics in grasslands devoted to livestock production in the context of increased nutrient loads in terrestrial ecosystems.

1 | INTRODUCTION

Human activities are increasing the density of domestic herbivores and global nutrient loads, modifying the main determinants of vegetation community dynamics (Oksanen et al., 1981; Gruner et al., 2008; Turkington, 2009). On the one hand, the composition of herbivores, the "top-down control" of vegetation biomass, has changed through the reduction of wild herbivore abundance (Ripple et al., 2015) and their replacement by domestic livestock with large mammals (Thornton, 2010). On the other hand, atmospheric contamination and the use of fertilizers have raised the local and global levels of plant essential nutrients, such as nitrogen (N) and phosphorus (P; Smil, 2000; Galloway et al., 2008), altering plants' resource availability, or the "bottom-up control" of vegetation production. Grasslands, in particular, have been deeply modified by human activities, as they concentrate the main areas of agriculture and livestock production globally (Hoekstra et al., 2005). This critically endangered biome accounts for approximately one-third of human appropriation of global net primary production (Krausmann et al., 2013). Despite its importance, we have scarce evidence about the mechanisms that explain plant biomass responses to simultaneous changes in resource availability and grazing in rangeland systems.

Grazing and soil nutrients generate opposing effects on plant community biomass (Gruner et al., 2008): grazing reduces plant above-ground biomass (Jia et al., 2018) while the addition of limiting nutrients increases it (Fay et al., 2015). However, it is not clear how the simultaneous presence of grazers and the addition of nutrients control plant biomass, either independently (Gruner et al., 2008) or interacting and modifying each other (Oksanen et al., 1981; Turkington, 2009). As a bottom-up control, fertilization increases nutritional quality of plant tissues (Firn et al., 2019) and promotes plant productivity (Fay et al., 2015; Tang et al., 2018). These changes, in turn, could stimulate plant consumption by grazers and consequently reduce plant biomass (Tripler et al., 2002; Augustine et al., 2003; van der Waal et al., 2016). At the same time, grazing, a top-down control, reduces standing plant biomass, which may lower light limitation to plant growth (Knapp & Seastedt, 1986; Borer et al., 2014b), thus enhancing plant productivity mainly in response to nutrient addition (Hautier et al., 2009; Gough et al., 2012). Therefore, several mechanisms can explain the interactive effects of grazers and nutrient inputs on plant biomass in grasslands, which remain unknown.

Most studies analyzing the joint effect of grazers and nutrients assessed only above-ground plant biomass (Augustine et al., 2003; Fariña et al., 2016; Frank et al., 2018), even though below-ground organs represent the predominant biomass pool in herbaceous systems (Poorter et al., 2012). Changes in plant allocation between above- and below-ground organs could be another mechanism to explain above-ground plant biomass responses to grazing or fertilization (Gao et al., 2008; Poorter et al., 2012; Wang et al., 2019). Previous studies showed that grazing increased (Piñeiro et al., 2009; López-Mársico et al., 2015), decreased (Gao et al., 2008), or did not modify below-ground biomass of grassland communities (Milchunas & Lauenroth, 1993; McNaughton et al., 1998). Grazing can also enhance root mortality, thus increasing below-ground turnover (Pucheta et al., 2004). The variable effects of grazing on below-ground organs are negatively modulated by defoliation intensity (Oesterheld, 1992; Zhou et al., 2017). There is also contradictory evidence about the impact of fertilization on below-ground biomass, with studies reporting positive (Ziter & MacDougall, 2013), negative (Cleland et al., 2019; Wang et al., 2019), or neutral effects (Liu & Greaver, 2010). Grazing and fertilization could also modify below-ground stratification (Piñeiro et al., 2009) and the allocation between acquisitive (roots) vs non-acquisitive (rhizomes, bulbs) below-ground organs (Klimešová et al., 2018). Consequently, the combined action of nutrient addition and grazing on below-ground biomass of plant communities remains unclear (Gough et al., 2012).

Despite its implications for grassland management, most of the studies that evaluated the importance of top-down and bottom-up controls on natural vegetation were carried out on freshwater and marine ecosystems, and the few studies performed in terrestrial ecosystems had no conclusive results (Gruner et al., 2008). In addition, previous studies in terrestrial ecosystems were performed under controlled conditions (growth chambers or pot experiments; Jaramillo & Detling, 1988; Georgiadis et al., 1989) and the grazing impact was assessed through mechanical defoliation (Lezama & Paruelo, 2016). The scarce studies in terrestrial plant communities that showed an interaction between herbivores and nutrients did not directly manipulate nutrient availability (Westoby, 1989; Augustine et al., 2003; Frank et al., 2018) or only reported above-ground biomass after plant consumption (Fariña et al., 2016), thus not accounting for the proportion of plant productivity consumed. A recent study from the Nutrient Network (NutNet), a coordinated experimental network in grasslands worldwide (Borer et al., 2014a), found that herbivores consumed the additional fertilization-induced biomass at sites with high grazing intensity (Borer et al., 2020). However, we still lack a comprehensive analysis of the mechanisms involved in this response, which requires the measurement of plant productivity, its nutritional quality, and the amount of plant biomass consumed by herbivores.

The temperate grasslands of the Flooding Pampa are the principal support of livestock production in Argentina (Viglizzo et al., 2011). Nevertheless, this region has a short evolutionary history of grazing by big mammals compared to other grasslands or savannas, which increases the potential for being altered by extensive cattle production (Milchunas et al., 1988). Previous studies had analyzed separately the short-term impact of domestic grazers, directly (Rusch & Oesterheld, 1997; Piñeiro et al., 2009) or simulated through mechanical defoliation (Semmartin & Oesterheld, 1996, 2001), and fertilization (Semmartin et al., 2007) on plant biomass in this region. However, to date, there is no information about long-term plant biomass responses to the combined effects of cattle and nutrient addition in these natural grasslands. In the present study, we directly manipulated the presence of grazers and soil nutrients in a grassland devoted to livestock production over several years, following the Nutrient Network protocol (Borer et al., 2014a). In this way, we studied how previously proposed mechanisms were involved in plant biomass responses to the joint effects of grazing and nutrient addition in natural field conditions, which have direct implications for the management of cattle production in temperate grasslands.

We hypothesized that the combined effects of nutrient addition and domestic grazing on plant biomass are not additive, but they modify each other through different mechanisms: (a) above-ground biomass consumption by grazers increases ground-level light availability, which in turn promotes the above-ground net primary productivity (ANPP) positive response to fertilization. By contrast, the ANPP response to fertilization will be negligible in the absence of grazers due to light limitation (the absence of a top-down control limits the bottom-up effects of nutrients on above-ground plant biomass). (2) Fertilization increases plant nutritional quality and ANPP which, in turn, intensify grazing consumption of vegetation. Thus, fertilization will magnify the reduction of above-ground plant biomass by grazers (the bottom-up control intensifies the top-down effects on above-ground plant biomass). (3) Above-ground plant biomass reduction is compensated by the increase in below-ground biomass (the bottom-up and top-down interaction modifies plant biomass allocation).

2 | METHODS

2.1 | Study system

The Flooding Pampa covers 90,000 km^2 in the province of Buenos Aires, east-central Argentina. The experiment was conducted at "Las Chilcas" ranch, 40 km south of Pila (36°16′ S, 58°15′ W; 15 m a.s.l.). The regional climate is temperate subhumid. Annual temperature range is 9.2°C in July and 21.2°C in January (Servicio Meteorológico Nacional, "Dolores Aero" station, www.smn.gob.ar). Rainfall during the experimental years (2013–2017, estimated from March of each previous year to February of the following year, prior to biomass harvest) was 1,038.5 ± 95.4 mm (mean ± SE), ranging from 751.4 mm in 2017 to 1,270.8 mm in 2015. Dominant soils are Typic Natraquolls

(Chaneton et al., 1996), with 3.1% organic matter and limited water infiltration (Lavado & Taboada, 1987). The flat topography and poor soil drainage contribute to the occurrence of floods during autumn–spring, while drought periods are common during summer (Lavado & Taboada, 1987). Mineralization rates of N and P were 17.36 g N m^{-2} year^{-1} and 1.59 g P m^{-2} year^{-1}, respectively (Chaneton et al., 1996). Livestock production promotes the accumulation of soil N in this grassland through urine and dung deposition, while it reduces the amount of P through cattle exportation (Chaneton et al., 1996). Grassland type corresponds to humid mesophytic meadows of the central Flooding Pampa, and comprises a species mix of forbs, legumes, and C3 and C4 graminoids (Perelman et al., 2001). The site has been grazed at 0.5 cattle units ha^{-1} for nearly 100 years, which is considered a normal stocking rate for this region. Current grazing management involves annual resting periods during winter and spring, and reintroduction of cattle from early summer to late autumn.

2.2 | Experimental design

To test the hypotheses, we established a factorial experiment of grazing exclusion and nutrient addition in a split-plot design in March 2013. The experiment was replicated in six blocks located at least 100 m apart from each other in one paddock of ~1,000 ha. Each block had two plots (main factor: ongoing grazing vs exclosure) and, within each plot, there were two subplots (subplot factor: control vs fertilization with NPK + micronutrients). So, each block consisted of four 25-m^2 subplots (Appendix S1). The six 20 m × 50 m exclosures for domestic grazing were established in 2004. In each grazing and exclosure area, subplots were randomly assigned to the fertilization treatments. At the beginning of fertilization (2013), nine years after the establishment of exclosures, live above-ground biomass was 344.21 ± 42.35 g m^{-2} in exclosures and 294.54 ± 28.88 g m^{-2} in the grazed grassland (mean ± SE) and the percentage of light reaching the ground was 3.6 times higher in the grazed grassland than inside exclosures. Fertilization consisted of the addition of 10 g m^{-2} year^{-1} of each nutrient, following the Nutrient Network experimental protocol (Borer et al., 2014a). Nutrients were applied in granulated formulations of urea (N), triple superphosphate (P), and potassium sulfate (K) three times a year, in May, September, and December. Only during the first experimental year (2013) a mix of micronutrients, including Fe, S, Mg, Mn, Cu, Zn, B, and Mo, was also applied with NPK fertilizer (Borer et al., 2014a).

2.3 | Data collection

To test our hypotheses, we sampled several variables in each subplot (n = 6 blocks). First, we measured growing-season peak above-ground biomass (g m^{-2}) in March (late summer) from 2013 (pre-treatment) to 2017 (fourth year of fertilization). We clipped all

standing plant material within two 0.2 m × 0.5 m frames, randomly located every year in each plot. We sorted biomass by senescence status in live and dead biomass (standing dead + litter). We dried all above-ground biomass samples at 62°C for 72 hr and weighed them (0.01 g).

To evaluate our first hypothesis, we measured ANPP during the growing season of the fourth experimental year (2017). We estimated ANPP as the increments between final and initial biomass of the live, standing dead, and litter compartments, respectively (from November 2016 to March 2017; Sala et al., 1981). We harvested above-ground plant biomass within one 0.2 m × 0.5 m frame in each plot in spring (November). At the same time, we installed mesh movable cages (60 cm long × 30 cm wide × 45 cm high) in the grazed fertilized and control plots. We removed the cages in March and harvested plant biomass inside and outside cages. We estimated growing-season ANPP in the grazing area using final harvest inside cages (Oesterheld & McNaughton, 2000). These estimations of growing-season ANPP in the study site were similar to previous annual ANPP values reported for this grassland (Sala et al., 1981; Semmartin et al., 2007).

We also recorded photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) above the canopy and at ground level in permanent 1-m^2 quadrats delimited inside each plot in March 2017, using a 1-m-long ceptometer (Cavadevices, Buenos Aires, Argentina). We estimated the percentage of light reaching the ground as the ratio of the ground-level PAR to ambient PAR multiplied by 100 (Borer et al., 2014b).

To test our second hypothesis, in November 2016 we harvested live above-ground plant biomass in all plots to assess the quality of plant tissue. We estimated N concentration (%) by the Kjeldahl method and P concentration (%) by the inductively coupled plasma atomic emission spectroscopy method (ICP-AES; LabSPA, CERZOS – CONICET, Buenos Aires province, Argentina).

To evaluate the differential grazing impact among control and fertilized plots, we estimated plant biomass consumed by herbivores as the difference between biomass inside and outside the same cages used to estimate ANPP in the grazed grassland, at the end of the growing season (in March 2017; Oesterheld & McNaughton, 2000). We also estimated grazing pressure as the ratio between biomass consumed by herbivores and ANPP.

Finally, to test our third hypothesis, we measured below-ground biomass (g m^{-2}) in March 2016. We sampled one 20-cm height × 6.5-cm diameter soil core per plot, located inside the frame where we clipped above-ground biomass that year. We subdivided the soil cores into two depths (0–10 cm and 10–20 cm), gently washed them with water on a mesh of 0.5 mm, and sorted below-ground biomass into roots, rhizomes, and bulbs. We were unable to separate live from dead below-ground biomass. We dried the material at 62°C for 72 hr and weighed it (0.001 g). We estimated above:below-ground biomass allocation as the ratio of above-ground biomass to total plant biomass of the year (sum of total above- and below-ground plant biomass).

2.4 | Statistical analyses

To analyze the joint effects of grazing and fertilization on the different response variables, we performed mixed-effect models (lme function in *nlme* package, Pinheiro et al., 2017) with R (R version 3.5.3, R Core Team, 2018). We modeled live and dead above-ground biomass as a function of grazing, fertilization, year, and their interaction as fixed-effect factors. The random structures consisted of blocks (*n* = 6), grazed and ungrazed plots nested within blocks (to represent the split-plot design of the experiment), and subplots nested within plots (to model the repeated measures: 2014–2017). We used initial pre-fertilization values (2013) of each variable as co-variables in these analyses.

To evaluate the cumulative effect over time of the interaction between grazing and fertilization, we analyzed live and dead above-ground biomass, below-ground biomass (total and its components: roots and rhizomes + bulbs), above:below-ground biomass allocation, growing-season ANPP, percentage of light reaching the ground, and leaf N and P concentration of the last experimental year as a function of grazing, fertilization, and their interaction (fixed effects). The random structure included blocks (*n* = 6) and plots nested within blocks. Biomass consumed by herbivores and grazing pressure were modeled as a function of fertilization as fixed effect and block as random effect. Live and dead above-ground biomass of the last year and root biomass were log-transformed, dead and rhizome + bulb biomass were log(1 + *X*)-transformed, and percentage of light reaching the ground, biomass consumed by herbivores, and grazing pressure were square-root-transformed. Residuals were normally distributed (Shapiro–Wilk test). We also modeled variance heterogeneity when necessary (varIdent function; Pinheiro et al., 2017). When interactions were significant (*p* < 0.05), we performed post-hoc comparisons through Tukey tests.

To conclude, we evaluated the direct and indirect (through ANPP, ground-level light, and leaf nutrient concentration changes) effects of grazing and fertilization, alone and in combination, on above- and below-ground plant biomass with a structural equation model (SEM; function psem in the *piecewiseSEM* package, which allows the fitting of small data sets; Lefcheck, 2016). The SEM accounted for the random structure of the experimental design (split-plot design; Lefcheck, 2016). The treatments were coded to represent their individual effect in relation to the exclosure without nutrient addition (reference level). In this way, "Fertilization" refers to the fertilized plots inside exclosures, "Grazing" refers to the unfertilized grazed plots, and "Graz: Fert" to the fertilized plots in the grazed grassland. We initially included all theoretically relevant paths for the data of the last experimental year (2017; Appendix S2). Below-ground data are from the third experimental year (2016), but we decided to include them in the model with the assumption that they remained constant until the end of the experiment. We trimmed the SEM, sequentially eliminating non-significant paths one by one, when this lowered the model's AIC (Akaike Information Criterion) by two units. The final model had the lower AIC and a non-significant *p*-value,
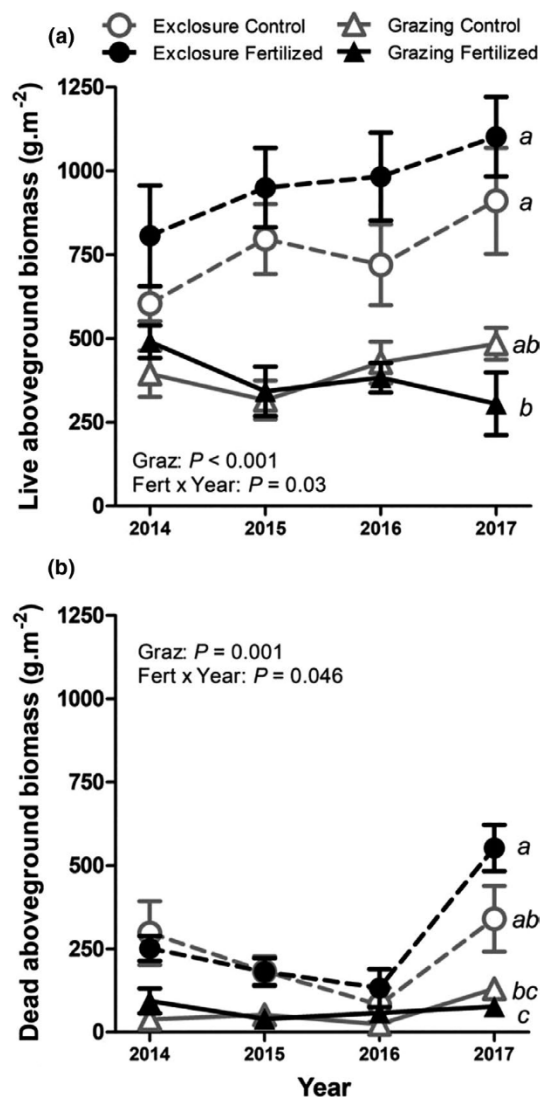
FIGURE 1 (a) Live and (b) dead above-ground plant biomass (g m^{-2}) during the four experimental years (2014–2017) in response to grazing (Graz), fertilization (Fert), and their interaction. Points are mean values ± SE; n = 6. Different letters denote significant differences during the last experimental year (2017) for live (Graz × Fert: $p = 0.04$) and dead above-ground biomass (Graz × Fert: $p = 0.02$)

which indicates non-detectable differences between the observed data and the prediction made by the SEM (Lefcheck, 2016).

3 | RESULTS

Grazing significantly reduced above-ground plant biomass during the four experimental years (Figure 1). The effect of grazing on above-ground biomass was marginally significantly different in response to fertilization (Appendix S3). Live biomass in grazed plots was 54% lower than inside exclosures for both fertilized and control plots (Figure 1a). Live biomass contributed 80% on average to total above-ground biomass. The remaining dead biomass was reduced by 75% by grazing (Figure 1b). During the last experimental year (2017),

fertilization reduced above-ground biomass under the grazing condition in relation to exclosures (Figure 1a, b, Appendix S3): grazing reduced live above-ground plant biomass by 52%, but when combined with fertilization this reduction was 70% (Figure 1a).

After four years, fertilization produced different effects on ANPP in exclosures and under grazing conditions (Figure 2a, Appendix S3). Inside exclosures, ANPP did not respond to fertilization, while in the grazed grassland ANPP increased by 103% with nutrient addition.

Light response to fertilization also differed among grazed and ungrazed plots (Figure 2b, Appendix S3). Inside the exclosures, fertilization reduced the percentage of light reaching the ground by 83%. By contrast, fertilization increased ground-level light under the grazing condition by 64%. The percentage of light reaching the ground was 32 times higher in the grazed grassland than inside the exclosures.

The nutrient quality of live biomass changed markedly with grazing and fertilization (Figure 2c, d, Appendix S3). Leaf N concentration increased on average 55% by grazing, compared with exclosure plots, and 23% by fertilization, considering the unfertilized plots of the grazed and ungrazed grasslands (Figure 2c). Fertilization increased leaf P concentration by 209% (Figure 2d), and there was no difference between exclosures and the grazed grassland. In the grazed grassland, fertilization produced a ten-fold increase in plant consumption by cattle (Figure 3a) and in grazing pressure (Figure 3b) compared with unfertilized conditions in 2017 (Appendix S3).

Neither total below-ground biomass nor its components (roots, rhizomes, and bulbs) significantly responded to grazing or fertilization (Figure 4a, Appendix S3). Total below-ground biomass consisted of 76% fine roots and 24% rhizomes and bulbs. Grazing reduced total below-ground biomass by 10% and increased rhizomes and bulbs by 60%, but these effects were marginally significant (Appendices S3 and S4). Below-ground biomass in the upper soil was concentrated in the first 10 cm (87%), and only 13% was present in the 10–20 cm soil depth. Plant biomass inside the exclosures was mainly allocated below-ground (above:below-ground biomass ratio below 0.50) and grazing reinforced this pattern (Figure 4b; Appendices S3 and S4). The above:below-ground biomass allocation did not change with fertilization.

Direct and indirect effects of the interaction between grazing and fertilization revealed by the SEM explained 74% of the variance on the above-ground biomass and 22% of the variance on the below-ground biomass (Figure 5, Appendix S2). Grazing decreased above-ground biomass, but this negative effect doubled when combined with nutrient addition (standardized estimate: −0.54 and −0.92, respectively; Figure 5). At the same time, grazing decreased ANPP (standardized estimate: −0.46) but increased leaf N concentration mainly in the fertilized plots (standardized estimates: 0.46 and 0.96, respectively), which indirectly promoted ANPP (standardized estimate: 0.55; Figure 5). This increase in ANPP fostered above-ground and reduced below-ground plant biomass by a similar magnitude (standardized estimates: 0.45 and −0.48, respectively). Fertilization, inside exclosures and in the grazed grassland, increased leaf P, while grazing, alone and combined with nutrient addition, promoted
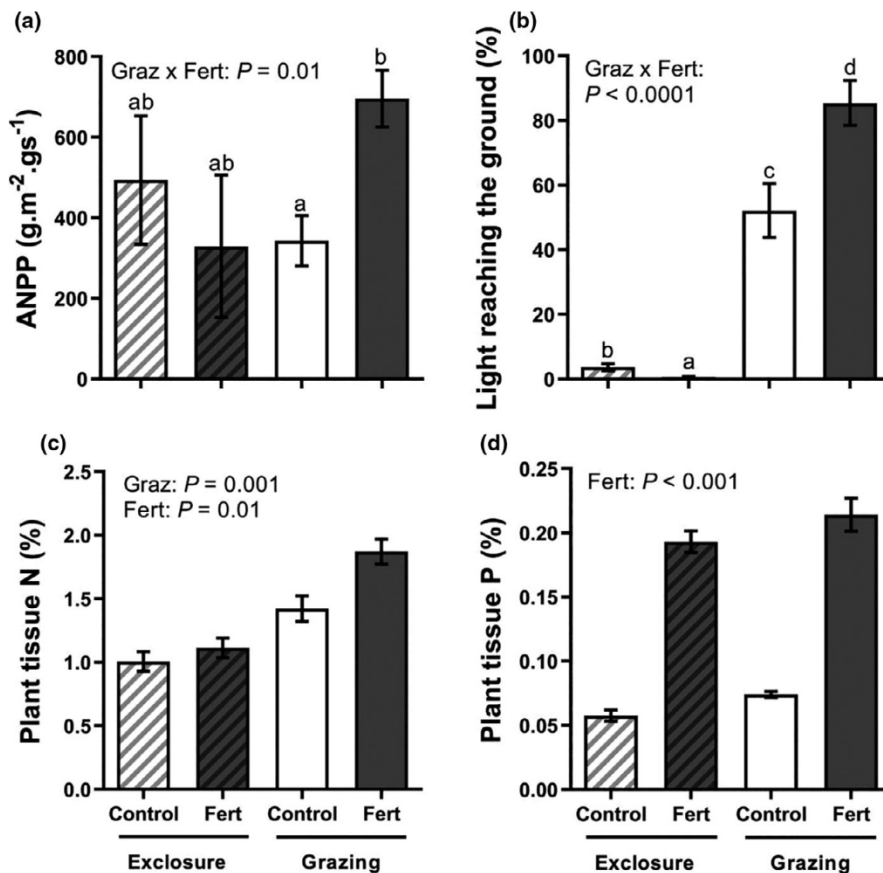
FIGURE 2 (a) Growing-season above-ground net primary productivity (ANPP; g m^{-2} gs^{-1}); (b) percentage of light reaching the ground; (c) plant tissue nitrogen (N); and (d) plant tissue phosphorus (P) concentration (%) during the last experimental year (2017) in response to grazing (Graz), fertilization (Fert), and their interaction. Bars are mean values $\pm$ SE; $n = 6$. Different letters denote significant differences among treatments when grazing by fertilization interaction was statistically significant ($p < 0.05$)

ground-level light, but these changes did not translate to modifications in ANPP or plant biomass (Figure 5).

4 | DISCUSSION

After four years, we found that the combined effect of grazing and fertilization interactively controlled grassland plant biomass. Grazing

FIGURE 3 (a) Growing-season domestic herbivore consumption (g m^{-2} gs^{-1}); and (b) grazing pressure (unitless) of the last experimental year (2017) in response to fertilization (Fert). Bars are mean values $\pm$ SE; $n = 6$

reduced above-ground biomass, but this reduction was greater when combined with nutrient addition (Figures 1 and 5). Additionally, grazing and fertilization in the grazed grassland indirectly promoted above-ground plant biomass through the increase in leaf N and ANPP (Figure 5). Fertilization had a positive effect on ANPP only in the presence of cattle, where there is higher ground-level light (Figure 2a, b). Grazers consumed the extra biomass produced in these fertilized patches (Figure 3), which also had a higher concentration of leaf N and P than the unfertilized plots (Figure 2c, d). Despite not finding direct effects of the interaction between grazing and fertilization on below-ground plant biomass (Figure 4), cattle indirectly reduced below-ground organs through their promotion of ANPP (Figure 5). This is the first study conducted in this region that evaluated the joint effects of cattle and fertilization on plant communities under field conditions over several years. Our results provided empirical evidence of the mechanisms that explained plant biomass responses to grazing and nutrient addition, which have direct implications for the development of sustainable management practices on temperate grasslands devoted to cattle production.

In partial support of our first hypothesis, grazing reduced above-ground plant biomass (Figure 1) and increased the percentage of light reaching the ground, under both fertilized and unfertilized conditions, whereas nutrient addition reduced ground-level light availability inside exclosures (Figure 2b). The accumulation of dead plant biomass inside exclosures, mainly in the fertilized plots (Figure 1b), might have caused the reduction in the ground-level light
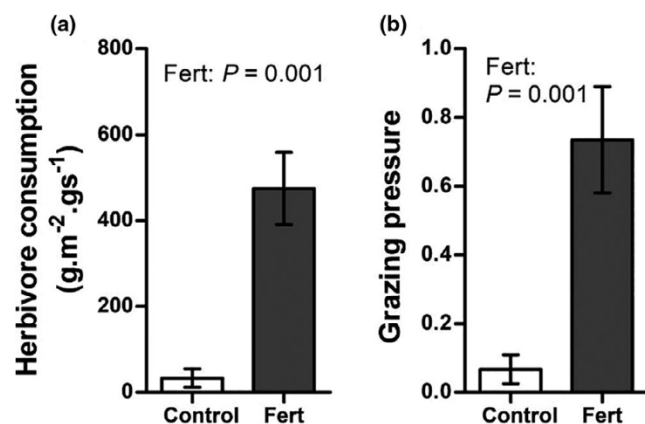
(Niu et al., 2010). This greater accumulation of dead biomass in 2017 might have been the result of the combined effects of the dry year and the life cycle of plant species inside the exclosures, which were not consumed by herbivores and died (Knapp & Seastedt, 1986; Niu et al., 2010). However, we did not find the hypothesized direct effect

of ground-level light on ANPP (Figure 5), which means that in this data set there is no causal relationship between light availability and ANPP. This contrasts with results from light-manipulative glasshouse experiments showing an increase in the fertilization effect on primary productivity with increases in light availability (Hautier et al., 2009). Thus, the interaction between grazing and fertilization in our work is not mediated by ground-level light but by leaf nitrogen (Figure 5), similar to what has been observed in previous studies (Hamilton III & Frank, 2001; Frank et al., 2018).

In agreement with our second hypothesis, we found that nutrient addition increased leaf N and P concentration (Figure 2c, d). Moreover, grazing pressure was higher in fertilized than in control plots (Figure 3b) and grazers consumed the extra biomass produced in these nutrient-rich plots (Figure 3a). Grazing intensification was the likely result of higher nutritional quality of plant biomass and higher ANPP (Augustine et al., 2003; van der Waal et al., 2016). Cattle selected and consumed proportionally more biomass in the fertilized plots, leading to a higher load in these nutrient-rich patches. Consequently, grazers neutralized the plant production induced by fertilization, which reduced above-ground plant biomass to a greater extent compared with unfertilized grazing plots (Figures 1 and 5). Therefore, the increase in plant nutritional quality due to the bottom-up effect of fertilization may explain the preference of grazers for the fertilized patches and the intensification of the top-down control on above-ground biomass (Tripler et al., 2002; Augustine et al., 2003; Cebrian et al., 2009; van der Waal et al., 2016).

We found that the impact of grazing in plant tissue N almost doubled the positive effect of fertilization (Figure 2c) and that grazing was the main control of leaf N concentration in our experiment (Figure 5). These results are consistent with previous studies of this grassland showing that plant species that increased their abundance in response to grazing have higher N tissue concentration and
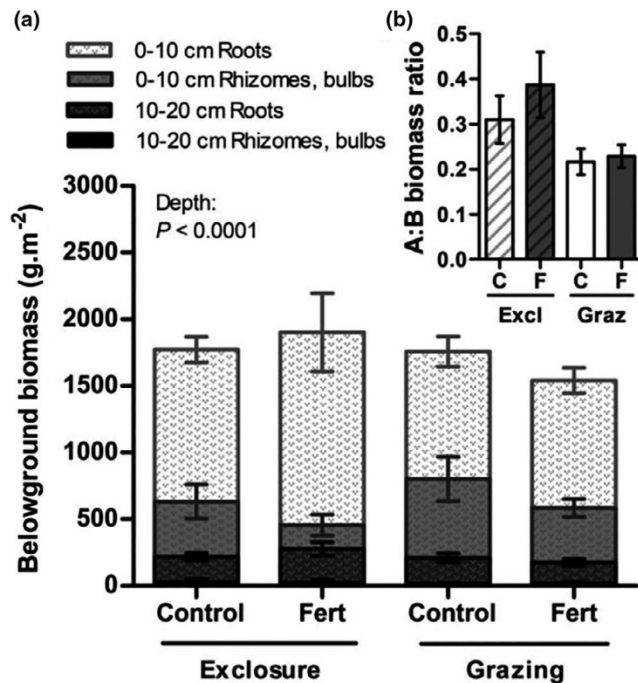
FIGURE 4 (a) Biomass of roots and rhizomes + bulbs (g m^{-2}) at different soil depths (0–10 cm and 10–20 cm); and (b) total above:below-ground biomass allocation after three experimental years (2016) in response to grazing (Excl or Graz), fertilization (Control [C] or Fert [F]), and their interaction. Bars are mean values ± SE; n = 6. The effects of treatments were not statistically significant (p > 0.05)
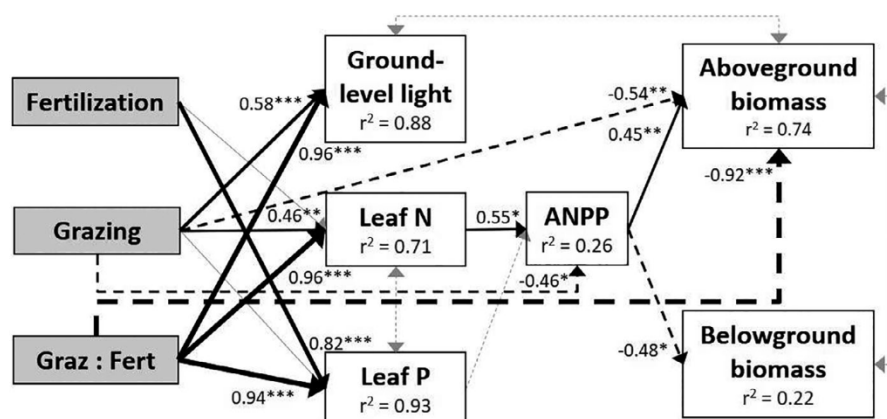
FIGURE 5 Final structural equation model of the effect of fertilization, grazing, and the combined effect of grazing and fertilization (Graz: Fert) on total above- and below-ground plant biomass (g m^{-2}) through ground-level light (%), leaf N (%), leaf P (%), and growing-season above-ground net primary productivity (ANPP; g m^{-2} growing-season^{-1}). The r^2 values within the boxes indicate the proportion of the variance explained by the fixed factors of the model for each response variable. The displayed estimates are standardized path coefficients. The width of the arrows reflects the strength of the respective positive (solid) or negative (dashed) path. Grey arrows show non-significant paths included in the final model. Significance levels of path coefficients are shown as * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Global model: Fisher's C = 32.72; p-value = 0.34; degrees of freedom (df) = 30

decomposition rates (Garibaldi et al., 2007). They also released more nutrients than those species that diminish with grazing (Garibaldi et al., 2007). Therefore, our findings suggest that plant compositional changes could mediate top-down and bottom-up controls on above-ground biomass (Fariña et al., 2016), which needs to be further explored. Grazers may accelerate nutrient recycling rates (Chaneton et al., 1996; Hamilton III & Frank, 2001; Singer & Schoenecker, 2003; Frank et al., 2018) and increase nutrient availability for plant growth (Figure 5). As cattle preferentially consumed biomass in the fertilized plots (Figure 3), there could be a positive feedback between consumption and plant nutritional quality (Augustine et al., 2003). Despite the lack of increases in plant biomass or ANPP with fertilization inside the exclosures (Figures 1 and 2a), leaf N and P concentration rose dramatically (Figure 2c, d), showing a "plant luxury consumption" (Tripler et al., 2002). Thus, the nutritional quality of plant biomass is probably mediating the interactive responses we observed in our study.

Finally, there is no complete support for our third hypothesis. Below-ground plant biomass showed a marginally significant reduction by grazing (Figure 4a, Appendix S4), which was indirectly mediated by the increase in ANPP (Figure 5). This result contrasts with previous studies performed at this site that found that the grazed grassland had a higher below-ground biomass than long-term exclosures (Piñeiro et al., 2009). Additionally, grazing tended to promote the biomass of rhizomes and bulbs (Figure 4a, Appendix S4), the below-ground organs that perform persistence and regrowth functions (Klimešová et al., 2018). The above:below-ground biomass ratio was also slightly reduced by grazers (Figure 4b, Appendix S4), but this was mainly due to the reduction in above-ground biomass and not by the increase in below-ground biomass. Therefore, the above-ground plant biomass reduction by cattle was not compensated by the increase in below-ground biomass, as expected (Poorter et al., 2012). A recent study from 29 grassland sites found that below-ground biomass increased with N addition only in sites with high ground-level light availability (Cleland et al., 2019), which may explain the lack of response of below-ground biomass to fertilization inside exclosures. Despite grazing effects on ground-level light, below-ground plant growth could be decoupled from the availability and uptake of soil resources (Kulmatiski et al., 2017). Nevertheless, studies conducted in other grasslands also found no effects of grazers on below-ground biomass (Milchunas & Lauenroth, 1993; McNaughton et al., 1998). Hence, our results suggest that domestic herbivores and fertilization interactively control above-ground, but not below-ground, plant biomass in the Flooding Pampa grasslands within the time frame of this study. The temporal scale of above-ground responses is generally faster than the below-ground one (van der Putten et al., 2009), so it is necessary to keep exploring the temporal dynamics of below-ground biomass through experiments involving longer time frames to understand above- and below-ground plant biomass allocation in response to grazing and fertilization.

In conclusion, nutrient addition and grazing synergistically controlled plant biomass in this temperate grassland. Grazing mediated plant responses to increasing soil resources. Domestic herbivores responded to the higher plant quality and quantity due to fertilization by preferentially grazing in fertilized plots, which led to a marked reduction in above-ground plant biomass. However, if domestic herbivores continue responding in the same way, plant composition will probably change in the long term due to overgrazing, promoting species resistant to consumption with lower nutritional quality (Li et al., 2017). These changes in plant composition are expected to intensify with time, and will depend on the stocking rate in relation to the paddock size, which will determine the evenness of grazing pressure across the paddock and the consequent changes of plant species composition and diversity (Bailey & Provenza, 2008; Pizzio et al., 2016; Porensky et al., 2017). In the present scenario of global change with increased nutrient inputs in terrestrial ecosystems, the intensification of the negative impact of grazers on plant biomass might produce undesirable consequences on grasslands and on the sustainability of livestock production. Understanding the mechanisms that modulate grassland plant biomass responses to grazing and increasing nutrient loads is a fundamental step toward the development of effective conservation and management strategies in rangelands worldwide.

AUTHOR CONTRIBUTIONS

SC and LY conceived the ideas, designed the study, and collected the data; SC analyzed the data and led the writing of the manuscript. Both authors contributed to successive drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All data generated during this study are available in the Supporting Information: Appendix S5.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. Experimental design

Appendix S2. Structural equation model information

Appendix S3. Results from mixed-effect models

Appendix S4. Natural log-response ratio (LRR) of below-ground biomass

Appendix S5. Data generated during the study